

RESEARCH LETTER

A gene cluster in *Agrobacterium vitis* homologous to polyketide synthase operons is associated with grape necrosis and hypersensitive response induction on tobacco

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Introduction

Agrobacterium vitis causes the debilitating disease, crown gall, on grapevines worldwide. *Agrobacterium vitis* is unusual among *Agrobacterium* spp. in that in addition to tumors it also causes necrosis of grape roots and a hypersensitive response (HR) on tobacco (Herlache *et al.*, 2001). The HR caused by *A. vitis* resembles HRs of other gram-negative bacteria (Alfano & Collmer, 1996): it is dependent on bacterial concentration, requires an active plant response, and results in the collapse of infiltrated tissue within 24 h. Unlike other bacteria that cause plant HRs, the genome sequence of *A. vitis* (<http://agro.vbi.vt.edu/public/>) does not encode a type III secretion system that is typically used for transfer of HR-associated effector proteins to plant cells.

Several chromosomal genes have been shown to be associated with HR and necrosis responses in *A. vitis*, including three transcriptional regulators belonging to the *LuxR* family, thus indicating the involvement of quorum-sensing (QS) regulation (Zheng *et al.*, 2003; Hao *et al.*, 2005; Hao & Burr, 2006). Mutation of *aviR*, one of the *luxR* homologs,

Abstract

Here, we identify a cluster of eight genes on chromosome 2 of *Agrobacterium vitis* that is associated with the ability of the bacterium to cause a hypersensitive response on tobacco and a necrosis of grape shoot explants. Three of these genes share a high level of structural and sequence similarity to clusters of genes in other bacteria that encode the enzymes for biosynthesis of polyketides and long-chain polyunsaturated fatty acids. No similar gene clusters were discovered in sequenced genomes of other members of *Rhizobiales*.

abolished HR and necrosis, indicating the sharing of a common pathway for both responses. In addition, a single gene related to the *N*-acyl-homoserine lactone (AHL) synthases, *avsI*, was shown to be involved in synthesis of long-chain AHLs that are required for regulation of HR and necrosis (Hao & Burr, 2006). Furthermore, a non-QS-related gene necessary for HR was identified (Herlache *et al.*, 2001). This gene, now called *avhF* (for *A. vitis* HR-associated genes) is similar to *pfaC* in marine bacteria (Okuyama *et al.*, 2007). In particular, bacteria such as *Shewanella* spp., *Photobacterium profundum*, and *Moritella marina*, carry well-characterized type I polyketide synthase genes for the production of the long-chain polyunsaturated fatty acids (LCPUFA) eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) (Yazawa, 1996; Tanaka *et al.*, 1999; Allen & Bartlett, 2002). Here, we report the presence of genes homologous to LCPUFA and polyketide synthases found within a gene cluster in *A. vitis*. The discovery of this cluster in *A. vitis* is of particular interest because it is comprised of genes not identified in other *Rhizobia* and suggests that fatty acid biosynthesis is associated with the

mechanism by which *A. vitis* is able to elicit the HR and necrosis responses.

Materials and methods

Bacterial strains, HR and necrosis assays

Agrobacterium vitis strain F2/5 was propagated on potato dextrose agar (PDA) (Difco Laboratories, Detroit) at 28 °C. F2/5 mutants were propagated on PDA or potato dextrose broth amended with 100 µg mL⁻¹ of kanamycin. *Escherichia coli* strains were propagated on Luria–Bertani (LB) agar amended with the appropriate antibiotics. Bacterial cultures for HR assays were grown for either c. 20 h or for 5 days on PDA or PDA amended with kanamycin. Cells were suspended in sterile distilled water at an OD_{600 nm} = 1.5 (c. 5 × 10⁹ CFU mL⁻¹), and infiltrated into tobacco leaves (*Nicotiana tabacum* cv. Xanthi) as described previously (Herlache *et al.*, 2001). HR was scored within 24 h after infiltration.

Necrosis assays were conducted using greenhouse-grown *Vitis vinifera* green shoot explants. Actively growing shoots were surface washed with a 50 : 50 (v/v) solution of bleach and sterile water for 20 min, washed in 70% ethanol for 5 min, washed with sterile distilled water, cut into c. 1-cm long sections, and placed vertically in 2% water agar plates with their basal ends up. The basal ends were then inoculated with 2 µL of 24–48-h cultures of bacteria suspended to an OD_{600 nm} = 1.0 (c. 1.0 × 10⁹ CFU mL⁻¹) and scored for necrosis over 3–4 days as described by Herlache *et al.* (2001).

Effect of bacterial supernatant on HR induction

To determine if a factor is secreted by *A. vitis* that facilitates HR induction, 20-h bacterial cultures were resuspended in sterile distilled water to an OD_{600 nm} = 1.5, centrifuged, and the resulting supernatant was passed through a 0.22-µm low protein-binding Acro disc (Gelman Sciences, Ann Arbor, MI). Cultures (20 h) of F2/5, and mutants of *avhE* and *avhF* were suspended to an OD_{600 nm} = 0.55 (c. 5.0 × 10⁸ CFU mL⁻¹) in filtered supernatants from either F2/5 or *avhE* or *avhF* mutants, and infiltrated into tobacco leaves (Table 1). The infiltrated leaf panels were scored within 24 h as: HR-positive (complete collapse of infiltrated area), partial HR (partial collapse of infiltrated area), or HR-negative (no collapse).

Cloning and sequencing of the Tn5-disrupted gene

The F2/5 mutants were created via transposon insertion as described previously (Herlache *et al.*, 2001). Genomic DNA was isolated using the UltraClean microbial DNA Isolation kit (Mo Bio Laboratories Inc., Solana Beach, CA) then digested with EcoRI, ligated into pBluescript SK⁻, and transformed

Table 1. Comparison of wild-type F2/5 and mutant supernatants on HR induction

Strain [†]	No. of leaf panels with different HR ratings*				
	Sup [‡]	SDW	F2/5	<i>avhE</i>	<i>avhF</i>
F2/5		0-1-20	32-1-0	17-5-1	17-3-1
<i>avhE</i>			0-0-17		
<i>avhF</i>			0-0-20		

*Table shows the results from experiments in which each treatment was repeated at least four times using leaves from different plants. The leaves were infiltrated with bacteria in sterile supernatant from F2/5, or *avhE* Tn5 mutant, or *avhF* Tn5 mutant. First number indicates a positive HR (complete collapse of the infiltrated area), the second number indicates a partial HR (partial collapse of the infiltrated area), and third number indicates HR negative (no collapse).

[†]Twenty-hour bacterial cultures were resuspended to an OD_{600 nm} = 0.55 in sterile supernatant from either F2/5, or *avhE* Tn5 mutant, or *avhF* Tn5 mutant.

[‡]Bacterial supernatants were prepared as follows: 20-h bacterial cultures were resuspended in sterile distilled water to an OD_{600 nm} = 1.5 and centrifuged. The supernatant was removed and passed through a 0.22-µm filter.

into *E. coli* JM109 cells. Transformants were selected on LB agar containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, (50 µg mL⁻¹), isopropyl-β-thiogalactopyranoside (100 µg mL⁻¹), kanamycin (100 µg mL⁻¹) and ampicillin (100 µg mL⁻¹). Plasmid DNA was isolated using the Rapid-Pure plasmid mini kit (Qbiogene, Carlsbad, CA) and sequenced at Cornell University DNA services (Ithaca, NY).

Site-directed mutagenesis

PCR and sequence analyses of genes within the HR-associated cluster in *A. vitis* strain S4 verified the presence of corresponding genes in F2/5. Targeted mutations in ORF1, and in *avhA*, *avhB* and *avhD–I* located in the HR-associated gene cluster were generated as described previously by Zheng *et al.* (2003). All the forward primers contained a SacI site and the reverse primers included an XbaI site for subcloning (Table 2). The PCR-amplified fragments were subcloned into the suicide vector pVIK165 (Kalogeraki & Winans, 1997) and introduced into the *E. coli* strain S17-1/λpir. After confirmation of the recombinant plasmid in S17-1/λpir, the construct was introduced into F2/5 by conjugation, which resulted in a single recombination event that disrupted the ORF of interest. The resulting mutants were selected on AB minimal medium (Chilton *et al.*, 1974) containing 100 µg mL⁻¹ of kanamycin. Mutants were confirmed by kanamycin resistance and PCR using a primer upstream of the insertion site and a primer within pVIK165. Visualization of *gfp* expression, a selectable marker on pVIK165, was performed using UV microscopy to further confirm the mutants.

Analyses of deduced protein sequence homologies were determined using protein BLAST (Basic Local Alignment Search Tool algorithm for protein sequences) provided by

Table 2. Primers used to disrupt ORFs and the HR phenotypes of resulting mutants

Gene	Primer sequence (5'–3')	Product size (bp)	Amplified nucleotides	HR*
<i>ORF1</i>	ATC <u>GAGCTC</u> GGCCGCTGATGGTGGGAAC [†] ATC <u>TCTAGA</u> CGGCGGCAAAATACAATCTCAAC [‡]	591	340–931	+
<i>avhA</i>	ATC <u>GAGCTC</u> GCTCATACTGGTCTGTCC [†] ATC <u>TCTAGA</u> GCTGCTTTCGATGGTAATG [‡]	615	500–1114	–
<i>avhB</i>	ATT <u>GAGCTC</u> GTAATATTGTTGTAACCGCAG [†] ATC <u>TCTAGAAA</u> CAGCCCACTCCATAC [‡]	214	52–266	–
<i>avhD</i>	ATT <u>GAGCTC</u> GCTACACCTCGGCGTTGG [†] ATT <u>TCTAGATT</u> CGAGACTGTGAGATTGG [‡]	631	107–738	–
<i>avhE</i>	GTA <u>GAGCTC</u> TATCGCAATTGTTGGTGTAG [†] AATTCTAGACTCGTCGAACGGCGAAATCAC [‡]	765	42–807	–
<i>avhF</i>	TCC <u>GAGCTC</u> CATATCCGAATTTGTGGTG [†] GGT <u>TCTAGA</u> GCGGTGGAAATGGTTGGTG [‡]	580	6218–6798	–
<i>avhG</i>	CTGG <u>GAGCTC</u> GCGATTCTTGCCCATAC TTGGTCTAGACGTTTTCTCGGCCACTTTCT	629	218–847	–
<i>avhH</i>	TCC <u>GAGCTC</u> ATCCGCTTGACGACGAC [†] ATT <u>TCTAGACG</u> TTTGCGACATTGACAC [‡]	630	8–638	–
<i>avhI</i>	ATC <u>GAGCTC</u> GCGGCATGATGTTTCTATC [†] ATC <u>TCTAGATC</u> GCGGCCAAGCACCTGAC [‡]	508	215–722	–

*HR was scored within 24 h after infiltration of bacterial suspensions into tobacco leaves.

[†]The underlined GAGCTC was added to create a SacI site for subcloning.

[‡]The underlined TCTAGA was added to create an XbaI site for subcloning.

the National Center for Biotechnology Information. Comparisons for putative protein domains were determined using the BLAST Conserved Domains algorithm with the threshold set to 1 and the low complexity filter turned off.

Analysis of gene expression

Reverse transcriptase (RT)-PCR was used to determine if the genes within the cluster are expressed as an operon. As described previously (Zheng *et al.*, 2003), total RNA was isolated from F2/5, and *avhE* and *avhF* mutants using the TRI-Reagent Kit (Molecular Research Center, Cincinnati, OH). RNA samples were treated with RNase-free DNase, checked for DNA contamination via PCR, and then subjected to RT-PCR using the One-Step RT-PCR system plus Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA). The products were separated by gel electrophoresis using a 1.2% agarose gel. Primers used for determining the expression of genes within the cluster are shown in Table 2.

AHL production

Because HR and grape necrosis phenotypes in *A. vitis* are associated with QS regulation AHL production by *avhE* and *avhF* mutants was compared with F2/5 as described previously (Zheng *et al.*, 2003). Overnight bacterial cultures (grown on PDA or PDA amended with kanamycin) were suspended in 20 mL of a 50:50 solution of acidified ethyl acetate–acetonitrile, shaken overnight at 150 r.p.m., and centrifuged at 11 800 g for 10 min. A second acidified ethyl acetate–acetonitrile shaken for 1 h at 150 r.p.m. Extractions

were dried by rotary evaporation followed by a stream of N₂. AHL profiles were determined using reverse-phase thin-layer chromatography overlaid with biosensor strain NTL4 (pZLR4) (Cha *et al.*, 1998).

Results

A large gene cluster is associated with HR and necrosis

Herlache *et al.* (2001) identified previously a gene in F2/5 (Avi5625 in strain S4) that is required for HR and necrosis and is homologous to *pfaC* found in bacterial LCPUFA operons (Okuyama *et al.*, 2007). Here, we report a Tn5 insertion in a homolog of *pfaA* (Avi5624 in S4), another gene found in bacterial LCPUFA operons. Both genes are required for 20-h cultures of *A. vitis* to cause an HR on tobacco and a blackened necrosis phenotype on grape shoots. (Fig. 1). The genes were named *avhE* and *avhF*, respectively. Although 20-h cultures of the mutants do not cause the HR, 5-day cultures were found to induce the response indicating that the product(s) of the cluster is associated with HR but not absolutely required (Fig. 1).

To determine whether the gene cluster encodes a secreted product that is associated with HR induction, bacterial cell concentrations were suspended in F2/5 and mutant supernatants and compared for HR. Table 1 shows that filtered supernatant from 20-h cultures of F2/5, *avhE*, and *avhF* support HR induction at significantly lower bacterial concentrations than cultures resuspended in water

(OD_{600 nm} = 0.55 vs. 1.5). Similar experiments were not done for the necrosis phenotype as this response is caused by lower cell concentrations and develops over a period of 3–5 days.

Partial sequences of *avhE* and *avhF* from F2/5 together with the genome sequence of strain S4 (<http://agro.vbi.vt.edu/public/>) were used to identify a cluster of eight genes: three that are homologs of typical marine bacterial LCPUFA synthases. Similar clusters have not been identified in other *Rhizobiaceae* that have been sequenced. All genes are

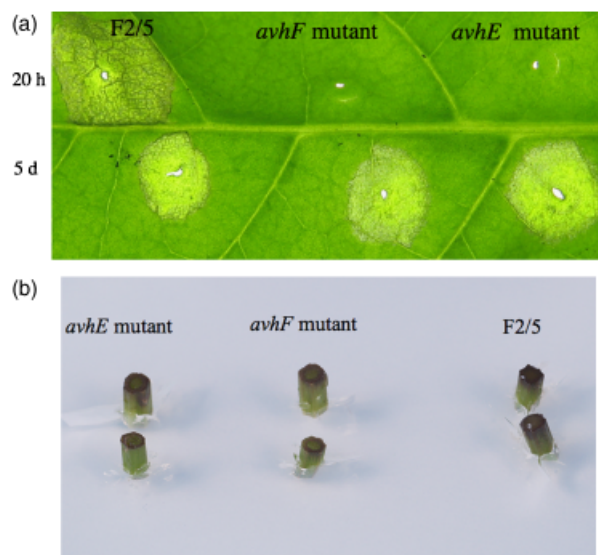


Fig. 1. Comparison of plant responses to wild-type F2/5 and mutants. (a) Twenty-hour cultures of F2/5 are HR-positive on tobacco leaves while the Tn5 mutants are HR-negative. All 5-day cultures are HR-positive. (b) F2/5 induces black necrosis on grape shoot explants; the Tn5 mutants show reduced brown necrosis induction.

oriented in the same direction, and the flanking genes show similarity to genes in *Rhizobiaceae*. The deduced protein product of the immediately upstream gene, ORF1, shows similarity to Atu4625 (84%) in *Agrobacterium tumefaciens*, and the deduced protein product of the immediately downstream gene shows similarity to YP_771481 (82%) in *Rhizobium leguminosarum*. Table 3 shows identity comparisons and putative gene products.

To further delineate the genes required for HR, site-directed mutations were introduced into *avhA*, *B* and *avhD–I*, and in ORF 1 (the ninth ORF in the cluster, *avhC* is very small and therefore was not tested). It was determined that all of the *avh* mutants are HR-negative on tobacco, while the flanking ORF1 is HR-positive (Table 1). PCR amplification and subsequent sequencing of selected segments of cluster revealed that the S4 and F2/5 sequence are highly similar, if not identical.

Comparison of the gene organization and protein functional domains

Three genes within the cluster, *avhE*, *avhF* and *avhG*, show a high degree of similarity to LCPUFA synthase operons of marine bacteria (Fig. 2). They show similarities in order, orientation, size and domain organization. The domain organization of putative AvhE is the same as PfaA except for slight differences in numbers of acyl carrier protein (ACP) domains. *Agrobacterium vitis* lacks a *pfaB* ortholog; however, AvhF contains an acyl transferase (AT) domain that is found in PfaB. Putative thioesterases are found adjacent to the LCPUFA orthologs in *A. vitis*, *P. profundum* and *M. marina*. Table 3 shows identity comparisons and putative gene products.

Table 3. Sequence similarity of *Agrobacterium vitis* gene cluster

ORF	Size*	Gene ID†	Similarity	Accession No.	Size*	% Identity	E-value
<i>avhA</i>	432	Avi5617	Amine oxidase (<i>Saccharopolyspora erythraea</i>)	YP_001102297	431	45 (189/411)	8e-105
			Amine oxidase (<i>Agrobacterium tumefaciens</i>)	AAK87735	457	35 (151/423)	4e-56
<i>avhB</i>	152	Avi5618	Integral membrane protein (<i>Beggiatoa</i> sp. PS)	ZP_02000949	190	37 (55/145)	9e-21
<i>avhC</i>	93	Avi5619	AMP-dependent synthetase and ligase (<i>S. erythraea</i>)	YP_001102298	95	54 (39/72)	6e-17
<i>avhD</i>	276	Avi5621	Hypothetical protein (<i>S. erythraea</i>)	YP_001102299	302	30 (80/262)	2e-15
<i>avhE</i>	2448	Avi5624	β-Ketoacyl synthase (<i>Shewanella oneidensis</i>)	AAN54658	2531	37 (956/2572)	0.00
<i>avhF</i>	2368	Avi5625	β-Ketoacyl synthase (<i>S. erythraea</i>)	YP_001102302	2322	39 (958/2423)	0.00
			β-Hydroxyacyl dehydratase (<i>Desulfatibacillum alkenivorans</i>)	ZP_02133172	2337	34 (835/2452)	0.00
			ORF7, (<i>Shewanella</i> sp.)	AAB81125	2004	37 (399/906)‡	6e-165
						34 (300/863)§	1e-129
<i>avhG</i>	526	Avi5626	PfaD (<i>Desulfatibacillum alkenivorans</i>)	ZP_02133173	537	52 (265/501)	9e-149
			PfaD (<i>Shewanella baltica</i> OS155)	YP_001049809	548	51 (260/509)	2e-147
<i>avhH</i>	548	Avi5627	Acyl-CoA dehydrogenase (<i>Burkholderia pseudomallei</i>)	ZP_02510024	586	28 (127/440)	4e-36
<i>avhI</i>	603	Avi5628	Acyl-CoA dehydrogenase (<i>S. erythraea</i>)	YP_001102294	591	32 (190/591)	3e-85

*Deduced size in amino acids.

†See <http://agro.vbi.vt.edu/public/>

‡Comparison with *Agrobacterium vitis* amino acids 2–824; note that AvhF show similarity in domain structure to PfaB and PfaC (see Fig. 2).

§Comparison with *A. vitis* amino acids 1542–2366; note that AvhF show similarity to both PfaB and PfaC (see Fig. 2).

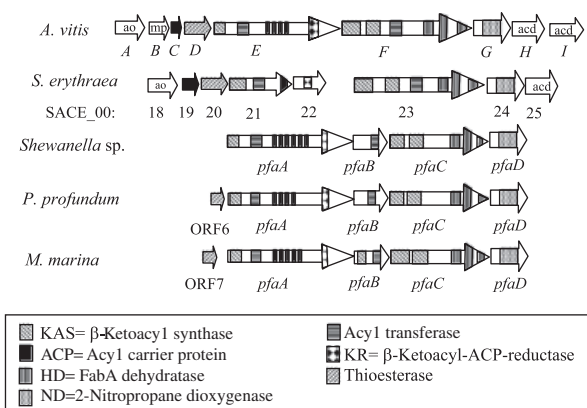


Fig. 2. Comparison of the putative *Agrobacterium vitis* gene cluster to the EPA and DHA gene clusters from marine bacteria and to a polyketide biosynthesis cluster in *Saccharopolyspora erythraea*. The arrow lengths represent the relative ORF sizes and direction of transcription. Gene number or name is given below each arrow. Mutants were created for all *avh* genes in the cluster except *avhC*. Domains that are not associated with LCP/PUFA are designated as follows: ao, amine oxidase; acd, acyl-CoA dehydrogenase; and mp, integral membrane protein.

Seven genes within the cluster are similar in organization, size and putative function to a cluster of polyketide biosynthesis genes found in the saprophytic soil bacterium, *Saccharopolyspora erythraea* (Table 3 and Fig. 2). This member of the *Pseudonocardiaceae* family is a gram-positive, filamentous bacterium that is used commercially for erythromycin A production (Oliynyk *et al.*, 2007). Noted differences between these clusters include the absence of *AvhB* in *S. erythraea* and an additional acetyl CoA dehydrogenase ortholog in *A. vitis*. A comparison of *AvhE* and SACE_0021 shows that *AvhE* is larger, carries four ACP domains instead of one, and has a β -ketoacyl-ACP-reductase domain that in *S. erythraea* is found in a separate ORF (SACE_0022).

The genes flanking the cluster show similarity to genes in *Rhizobiaceae*. The deduced protein product of the immediately upstream gene, ORF1, shows similarity to Atu4625 (84%) in *A. tumefaciens*, and the deduced protein product of the gene immediately downstream shows similarity to YP_771481 (82%) in *R. leguminosarum*.

avhE and *avhF* are expressed as an operon

Gene expression within the cluster was compared in F2/5 and the *avhE* and *avhF* mutants (Fig. 3). Expression of all genes was evident except for *avhE* and *avhF* in both of the mutants, indicating that they are coexpressed.

The effect on AHL profiles

Gene mutants within the cluster were not affected in production of AHLs as compared with F2/5 (not shown);

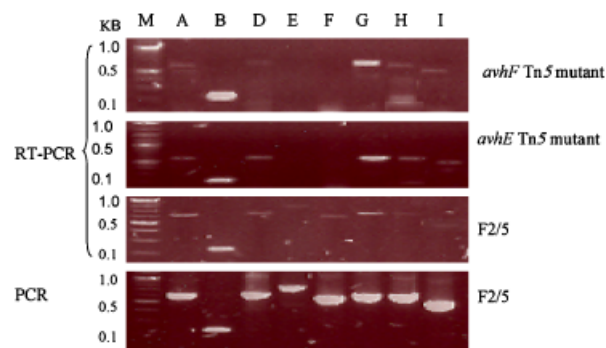


Fig. 3. Detection of gene expression in an HR-requiring gene cluster in *Agrobacterium vitis* and its mutants, *avhE* and *avhF* using RT-PCR. M, DNA marker; A, B, D, E, F, G, H, and I corresponds to *avhA*–*avhI* in the gene cluster. Interrupting either *avhE* or *avhF* prevents the expression of the other gene, showing that *avhE* or *avhF* are coexpressed.

therefore, the encoded products from this cluster do not appear to be associated with production of AHLs.

Discussion

A cluster of eight genes in *A. vitis* that is associated with ability to cause a tobacco HR and grape necrosis was identified. It is particularly interesting that the cluster has not been identified among the 12 other members of *Rhizobiales* that have been fully sequenced. *Rhizobia* inhabit a broad range of environments and include mammalian pathogens (*Brucella* species), plant pathogens (*Agrobacterium* species) and symbiotic nitrogen-fixing bacteria (*Rhizobium* species and *Mesorhizobium loti*). The fact that none contain a similar cluster suggests that *A. vitis* may have acquired the cluster via lateral gene transfer, although there is no evidence of insertion elements in the flanking regions of the gene cluster. In addition, the GC content of the cluster ranges from 51% to 61%. Although this varies from the rest of chromosome 2 (57.5), it is not by itself convincing of lateral gene transfer (Ochman *et al.*, 2000).

Agrobacterium vitis avhE–*G* show similarity to marine *pfaA*–*D* genes. DHA and EPA synthesis in marine bacteria require five genes; the products of four genes (*pfaA*–*D*) are directly involved in EPA or DHA synthesis while the product of *pfaE* is responsible for posttranslational modification of *PfaA* (Yazawa, 1996; Tanaka *et al.*, 1999; Metz *et al.*, 2001; Allen & Bartlett, 2002). The deduced proteins of *A. vitis avhE*, *F* and *G* show similarities in size and domain structure to marine bacterial *PfaA*–*D* proteins, most relevant, they carry the six common domains that are also found in *PfaA*–*D*: ACP, AT, β -ketoacyl synthase (KS), β -ketoacyl-ACP-reductase (KR), FabA dehydratase (HD), and 2-nitropropane dioxygenase (ND). Another bacterium, *S. erythraea*, appears to be the only other reported land bacterium that

shares homology with *pfaC* and *pfaD*; however, *S. erythraea* SACE_0021 is quite different from the marine bacterial PfaA and *A. vitis* AvhE. SACE_0021 is smaller and shares a similar domain structure to other polyketide synthase genes in *A. vitis* and *A. tumefaciens* (Avi 7299, Avi 7309, Atu 3681, and Atu 3672; <http://agro.vbi.vt.edu/public/>). The operon structure of *avhE–G* is similar to those in marine bacteria. In *P. profundum*, *pfa A–C* are expressed as an operon (Allen & Bartlett, 2002). *Agrobacterium vitis avhE* and *avhF* (whose deduced proteins carry the same domains as PfaA–C) are also coexpressed, while *avhG*, like *pfaD*, is expressed independently.

Due to similarities in domain and operon structure between *avhE*, *avhF* and *avhG* and marine LCPUFA genes and other polyketide synthases, it is possible that *A. vitis* produces a LCPUFA or a polyketide. *Agrobacterium vitis* produces a variety of unsaturated fatty acids, the most prominent being 18C unsaturated fatty acids (18:1 ω 7*cis*/18:1 ω 9*trans*/18:1 ω 12*trans*, 18:1 ω 7*cis*/18:1 ω 9*cis*/18:1 ω 12*trans*) which make up between 64% and 74% of the total fatty acid composition (Bouzar *et al.*, 1993; Jarvis *et al.*, 1996; Tighe *et al.*, 2000). In addition, *A. vitis* also produces several other long-chain fatty acids, including: 16:1 ω 7 *cis*-(palmitoleic acid), 18:0, 18:1 2-OH, 19:0 10-methyl, and 20:3 ω 6 *cis*-(dihomo- γ -linolenic acid). Fatty acid analysis of *A. vitis* strain F2/5 and the Tn5 mutant of *avhF* are very similar except that only the *avhF* mutant produces 12:1 3OH, and the mutant has a higher percent of the fatty acid 10:0 3OH (11.46 vs. 5.04) than wild-type strain F2/5 (T.J. Burr, unpublished data). The lack of 12:1 3OH in F2/5 suggests that this fatty acid may represent interrupted polyketide synthesis in the *avhF* mutant.

HR and necrosis induction by *A. vitis* is a complex system that involves QS regulation and the *avhA–I* cluster reported in this paper. QS regulates a variety of physiological processes in gram-negative bacteria, including symbiosis, biofilm formation, antibiotic production, virulence and disease (Whitehead *et al.*, 2001). *Agrobacterium vitis*-induced HR and necrosis involve at least three *luxR* homologs (*aviR*, *avhR*, and *avsR*) and one *luxI* homolog, indicating a complex system of QS regulation that involves several interacting branches. We determined that the *avh* gene cluster does not affect AHL production and does not appear to be regulated by QS. In addition, a secreted factor facilitates HR because 20-h culture supernatants support HR at lower bacterial concentrations. The *avh* gene cluster does not appear to affect the production of the secreted factor because *avhE* and *avhF* mutant supernatants usually support lower bacterial concentrations, and because the F2/5 supernatant does not complement either mutant.

Although the products of *avh* gene cluster are not required for HR because 5-day cultures cause the reaction, it appears that they may synthesize an HR and necrosis-enhancing factor. Polyketides play a role in pathogenesis in

other pathogenic bacteria. For example, *Pseudomonas syringae* pv. *tomato* DC3000, produces the phytotoxin coronatine which is composed of a coronafacic acid (a polyketide) and cyclopropyl amino acid. While coronatine is not essential for pathogenesis (Bender *et al.*, 1999), it is responsible for suppression of plant defense responses (Brooks *et al.*, 2005; Uppalapati *et al.*, 2007). Because the *avh* mutants cause reduced necrosis, the product of this culture may also reduce host plant defenses, while enhancing nonhost defenses. Further research is needed to characterize the products of the *avh* cluster and to determine the role of gene products in the mechanism of HR and necrosis.

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